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BIOLOGICAL BULLETIN

NOTES ON THE EFFECT OF X-RADIATION ON THE DEVELOPMENT OF CUMINGIA EGGS.[1]

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The energy of X-rays and of radium rays has been used by a number of investigators during recent years for modifying the normal course of development in living eggs. It has been sought to show by this means what efficacy these rays possess in altering vital properties of protoplasm, and to gain additional information concerning the normal as well as the abnormal reactions of living matter. One of us (Richards, 1914) had studied the effects of X-radiation upon early cleavage and development of *Planorbis*, a gasteropod, and it seemed probable that *Cumingia*, a lamellibranch, would offer interesting comparative data as well as give results of importance in themselves. For this reason the experiment herein described was performed.

Cumingia tellinoides is a form the eggs of which are very frequently used for experimental purposes at Woods Hole. The eggs and sperm are easily obtained separately, and may therefore be subjected to radiation either before or after fertilization.

The normal development of *Cumingia* is not as well known as

[1] During the month of August, 1914, while working at the Marine Biological Laboratory at Woods Hole, the senior writer performed experiments to test the effect of x-radiation upon the eggs of *Cumingia*. This experiment yielded results which would have justified a much more extensive study of the behavior of these eggs under the stimulation of x-radiation, but circumstances have prevented its repetition. In spite of the fact that the experiment has not been followed up, and that the number of eggs worked upon was not large, it has been decided to make note of the results obtained, for they extend our knowledge of radiation effects.

During the course of the experiment eggs were preserved (usually in Bouin's fluid) for later cytological study. This material has been sectioned and studied in the Zoölogical Laboratory of Wabash College.

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is that of some other forms, but the more important features are described by a number of writers. Morgan, Jordan, Morris and others in their descriptions of experiments have presented normal conditions as well as results of experiments.

In *Cumingia* eggs, Morgan ('10) found that normal development may follow in eggs that have undergone very considerable disturbances during experimental treatment, even "when the visible substances are unequally distributed, and are carried over into the blastomeres, redistribution being thereby prevented." Cleavage is persistently normal under very abnormal conditions, although very many factors may induce abnormal development, and the results manifest themselves only later. Abnormalities are especially apt to be caused by rough handling of the eggs in sea water after laying, injury to the egg membranes apparently being attended with serious consequences. "None of the visible substances are essential to the development of special parts of the embryo."

Polyspermy occurs in *Cumingia* eggs generally unless the sperm suspension be diluted before fertilization. This commonly is the cause of abnormal embryos, if it is permitted to take place to any extent at all. In our experiment the number of extra sperm, while not large, varied definitely depending upon the dosage of the X-rays, and therefore served a useful purpose in giving additional information as to the effectiveness of the rays.

The breeding season of *Cumingia* is at its height late in June and during the month of July. In August it declines and fewer eggs are laid by each female. The general impression prevails also among those who have worked with this form most that at the end of the season the egg is more easily injured, for instance by physical treatment, and so the percentage of eggs of a given lot which develops is less at that time. The number of eggs at any time which will develop depends upon their manipulation, for unnecessary handling with pipettes will cause low ratios.

At the time of fertilization the egg is in the metaphase of the first maturation division. Upon fertilization, maturation is completed in the usual manner.

The form and size of the first cleavage division is very constant and definite. The first division is unequal, the CD cell being

much larger than the AB cell (Morris). The CD cell divides before the AB producing a 3-cell stage, then a 4-cell. After the third division cleavage becomes irregular, the D cell, or its derivatives, remaining for some time distinctly larger than the other blastomeres.

EXPERIMENTS.

In the set of experiments here recorded, all exposures to the X-ray tube were made at the same time; thus all three-minute irradiations were under the tube simultaneously All three-minute irradiations were removed at the same time, as were all fifteen-minute irradiations. Consequently there is in all the data presented no question whatever as to any varying amount of exposure for the same length of irradiations.

The irradiation intensity was therefore, a constant; hence, we may neglect it entirely and take the time in minutes as a measure of the amount of irradiation, not concerning ourselves quantitatively with the physical units involved. This is a much simpler method since when one attempts to calculate the amount of irradiation he enters a field entirely beyond the scope of this paper. What we wish to show is the comparative effect of different amounts of irradiation when conditions are identical.

Heat, the dilution of the suspension, and the other external factors were kept constant in order to neglect them and reduce any possible chance for error by a faulty technique or varying conditions.

Four series of experiments were set up. In the A series the eggs were irradiated immediately after fertilization; in the B series, sperm were radiated and then used to fertilize normal eggs; in the C series, eggs were radiated and then fertilized by normal sperm; in the D series, both egg and sperm were radiated before fertilization.

The first experiment was carried on to test the effect of short and long irradiations on the freshly fertilized egg. For this test fresh sperm and eggs were obtained from the individual animals and immediately mixed. Fertilization took place immediately. This lot was divided into three parts, one for control and two for experiment. All were allowed to develop under similar conditions. Samples of the three lots were fixed at intervals during

the maturation and early cleavage divisions. A tabulated form of the observations is shown in Table I.

TABLE I.

Time After Irradiation.	Stage of Development.		
	Control.	A_3 Radiated 3 Minutes.	A_{15} Radiated 15 Minutes.
20 minutes.	Completing 1st maturation division.	In second maturation division 1st polar body present.	Beginning 2d maturation division.
43 minutes.	40% in 2-cell stage rest dividing in 1st cleavage division.	Completing 1st cleavage division.	5% in 2-cell stage rest dividing.
60 minutes (1 hour).	36% in 2-cell stage. 14% in 4-cell stage.	25% in 2-cell. 25% in 4-cell.	30% in 2-cell. 10% dividing. 10% in 4-cell.
80 minutes ($1\frac{1}{3}$ hours).	25% in 2-cell. 17% in 4-cell. 8% in 8-cell.	20% in 2-cell. 20% in 4-cell. 10% in 8-cell.	25% in 2-cell. 20% in 4-cell. 5% in 8-cell.
325 minutes (5 hrs. 25 min.).	17% of those developing are free swimming larvæ.	10% of those developing are free swimming larvæ.	1% of those developing are free swimming larvæ.
385 minutes (6 hrs. 25 min.).	———	———	10% of those developing are free swimming larvæ.

Radiation at first serves to stimulate the mitotic activity of these eggs. The results are not as clear cut as in *Planorbis* where entire egg clusters can be observed without in any way disturbing the eggs in their normal environment. Nevertheless, it is clear here from Table I. that acceleration takes place at first in the irradiated eggs and that subsequent retardation follows as in the case of *Planorbis* where "the control, started at the time of the exposure goes more slowly than the experiment during the first two mitoses, but by the time the twenty-four-cell stage is reached the exposed eggs are progressing more slowly than it" (Richards, '14). Packard ('16) obtained similar results in *Arbacia*.

In a cluster of *Planorbis* eggs division takes place almost simultaneously in all the eggs. No such degree of uniformity is to be observed in *Cumingia*, but the data clearly indicate that the acceleration, while it persists longer than in *Planorbis*, just as surely gives way to a retardation, and that the longer radiation causes a greater retardation than the shorter. It would

appear that greater exposure caused greater stimulation at first than the shorter, agreeing in this particular with the marine eggs which Packard ('16) studied, rather than with the fresh-water *Planorbis* and *Physa*.

From the 43-minute stage on the radiated eggs become slower in divisions, the percentage of free swimming larvæ in A3 being only a little over half the per cent. in the control, 325 minutes after fertilization. Only one or two per cent. of the A15 set have reached the free-swimming larval stage. However, from Table I. we see that one hour later or 385 minutes after fertilization it showed approximately the same percentage as the A3, 385 minutes after fertilization. This shows A15 at 325 minutes after fertilization is 20 per cent. behind A3 and approximately 35 per cent. behind the control in respect to time.

In figuring percentages of gain or loss during development and cleavage, we, following Packard, divide the difference in time required for the two sets to reach a certain stage by the time required by the control to reach that stage. Thus in the A series the control passed the 10 per cent. free-swimming larval stage several minutes ahead of A3. Now A15 entered that stage just 60 minutes later which is 20 per cent. retardation when compared with A3.

It was deemed inadvisable to carry the experiment farther for in both the control and the radiated lots a marked disintegration set in. It has been shown that disintegration may follow exposure to either radium (Packard) or X-rays (Richards, '15), but our radiation is hardly to be held responsible for the results of this experiment, since both control and experiment show it equally.

In Table I. reference is made to the percentage of the entire number of eggs which developed in the experiments. At the height of the breeding season a very large percentage of the number of *Cumingia* eggs fertilized are found to develop, usually over 95 per cent., it is stated. The precaution must be taken in that case to use dilute sperm in order to prevent polyspermy. The eggs used in this experiment, however, were obtained late in the summer and some polyspermy was permitted, for it was found, as will appear later, that the number of sperm entering

the egg is itself influenced by irradiation. A count, made as carefully as possible, of the eggs of this experiment gives 54.25 per cent. as the average number developing. The data given in the tables refer to the number of eggs which developed, not to the total number of the experiment. These numbers were verified by counts of sections as well as of the entire eggs. Of the sections counted 53.5 per cent., and of the whole eggs, 55 per cent. were found to be developing, giving an average of 54.25 per cent.

The B, C and D series were governed by one control. Since all irradiations and fertilizations were at the same time this was made possible. Experiment B was performed to test the effect of two different lengths of exposure when sperm were irradiated and used to fertilize normal eggs. They were subjected to 3- and 15-minute exposure as in the A set. Samples of the control and of the experiment were fixed at varying intervals after fertilization. A study of these samples gave the following results:

TABLE II.

Time After Irradiation.	Stage of Development.		
	Control.	B_3.	B_{15}.
21 minutes.	Completed first maturation division.	Completing 1st maturation division.	Completing 1st maturation division.
31 minutes.	Early prophase of 1st cleavage division.	20% completing 2d maturation divisions and others developing are in the prophase of 1st cleavage division.	Completing 2d maturation division.
74 minutes.	Pro- and metaphase of 1st cleavage division.	Same as control.	Prophase of 1st division.
381 minutes.	20% of those developing are free-swimming larvæ.	25% to 30% of those developing are free-swimming larvæ.	10% of those developing are free swimming larvæ.

From this table we see that 20 minutes after fertilization the first sample shows no difference whatever between control and irradiated sets. The second sample taken 10 minutes later shows no apparent difference yet in the sets. The third sample, 74 minutes after fertilization, shows the short irradiation still the same as the control while the 15-minute irradiation is slightly behind. There is a marked difference in the percentages of

free-swimming larvæ 381 minutes after fertilization. B3 shows an increase, B15 a decrease over the control.

Several observers have noted a stimulation to cell division when eggs have been fertilized by sperm subjected to an exposure of short duration. This observation can now be recorded for *Cumingia*. It is unfortunate that we have no samples of the experiment between the 74-minute and 381-minute stages of development in order to trace this through the cleavage development. The longer irradiation shows the expected retarded development, this length irradiation being harmful.

The C series consisted of radiated eggs fertilized by normal sperm. As stated before the control is the same as for series B. The following is a tabulated result from a study of the samples taken.

TABLE III.

Time After Irradiation.	Stage of Development.		
	Control.	C3.	C15.
21 minutes.	Completed first maturation division.	Giving off 1st polar body.	Slightly behind C3.
31 minutes.	Early prophase of 1st cleavage division.	In second maturation division, 1st polar body present.	In second maturation division.
74 minutes.	Pro- and metaphase of 1st cleavage division.	Early prophase of 1st division.	Same as C3.
381 minutes.	20% of those developing are free-swimming larvæ.	Nearly all dead; only one or two swimming larvæ.	Nearly all dead.

From this table it is evident that even a slight irradiation of the unfertilized egg causes retardation in the rate of cleavage and the development. That they do not develop shows that something has been interfered with in the metabolism of the embryo. Fertilization, however, appears normal in every respect and the polar bodies are given off normally.

The D series consisted of radiated eggs fertilized by radiated sperm. The control was the same as for B. Samples were taken similar to the other series. A study of the samples gave the following results.

Fertilization and maturation are slower in both irradiated sets than in the control. The shorter radiation, 74 minutes after fertilization shows a retardation over the control. The 15 minute

TABLE IV.

Time After Irradiation.	Stage of Development.		
	Control.	D_3.	D_{15}.
21 minutes.	Completed first maturation division.	35% giving off 1st polar body.	Same as D_3.
31 minutes.	Early prophase of 1st cleavage division.	10% in prophase of 1st cleavage division rest completing 2d maturation.	All completing 2d maturation.
74 minutes.	Pro- and metaphase of 1st cleavage division.	Early prophase of 1st cleavage division.	2d polar body present; no prophase of 1st cleavage division.
381 minutes.	20% of those developing are free-swimming larvæ.	Nearly all dead; one third of living are free-swimming larvæ.	Nearly all dead; one sixth of living are free-swimming larvæ.

irradiation is behind the 3-minute exposure. The same handicap is present here as in the two previous series; there being no samples after this period until the free-swimming larval stage is reached. Results of development, 381 minutes after fertilization, however, show that the effect is quite similar to the series where the egg only has been irradiated.

It is worthy of note that here, as in the cases reported by the Hertwigs and by Packard, that the rays produce more effect upon the fertilized eggs than upon the unfertilized. (Compare Tables I. to IV., the A and the B, C, D series.) In general the effect of a short irradiation of the fertilized egg of *Cumingia* is a stimulation in the rate of cell division through the first and second cleavage divisions, after which time there is a retardation. A longer irradiation causes a less acceleration than the shorter treatment and markedly greater retardation and inhibition of growth. In the case of a short irradiation of the sperm (B series) no change whatever can be noted in the rate of division. In the C series where the unfertilized egg is subjected to a short irradiation, the effect after fertilization is a direct retardation of development and a complete inhibition of growth by the time the free-swimming larval stage is reached. There is apparently only a difference of degree in the extent of the injuries due to the two strengths of exposure.

The result of the D series is quite similar to that of the C

series. It is evident that the irradiation of the sperm (B series) is not so harmful as a similar irradiation of the egg.

From a comparison of the number of eggs developing in the different series to that of the control a striking difference is noted. The following table will give by actual count the percentage of eggs developing in the different lots. We find no evidences in the nuclear structure of parthenogenetic development.

TABLE V.

Lot.	Percentage Developing.	Percentage Above Normal.	Percentage Below Normal.
Control (average)	54.25		
B3	60	5.75	
B15	50		4.25
C3	40		14.25
C15	40		14.25
D3	35		19.25
D15	35		19.25

For a sperm exposure of 3 minutes there is a net increase of almost 6 per cent. of the eggs developing. That is, there is a greater number of fertilizations due to a short exposure of the sperm. A longer exposure shows a decrease in the percentage of fertilizations which is below normal. The C series shows a net decrease of 14 per cent., there being no difference evident between the lots of eggs radiated for the longer or the shorter period. The D series is not, as might be expected, an average of the effect of B and C, but is a further decrease, the increase in the B series not being manifested in the D series. In fact the D15 eggs show a deviation from normal nearly equal to the sum of the deviations of the B15 eggs plus that of the C15 lot.

It appears, then, if this single set of observations be regarded as typical, that the percentage of *Cumingia* eggs fertilized is influenced by exposure of the eggs or sperm to X-radiation, and by the amount of exposure which they receive.

In connection with the effect of irradiation upon the percentage of fertilizations is the question of the effect upon polyspermy, for the same factor which would increase the percentage of fertilization would also tend to increase the number of supernumerary sperm to enter the egg. The results of actual counts

of the number of extra sperm in the sections studied are presented in Table VI.

TABLE VI.

Lot.	Percentage Developing.	Extra Sperm.	No. Above Control.	No. Below Control.
Control (average)	54.25	1.59		
B3 (average)	60	3.40	1.89	
B15	50	1.20		.39
C3	40	2.58	.99	
C15	40	1.46		.13
D3[1]	35	1.00		.59
D15	35	1.50		.09

Fertilization, of course, is not simply a selection by the egg of one sperm nucleus out of several, for if that were the case it would follow that the increase in the number of sperm to enter the egg would insure a higher percentage of fertilizations. There is no definite relation between the number of eggs fertilized and the number with more than one sperm. B3 has the greatest number of extra sperm, and is also the highest in number developing; C3, however, is next in number of extra sperm, but the percentage of the eggs in development is below the control. These results show, since columns one and two of this table are inconsistent with each other, that at least one other factor (and, of course, many factors) are involved in fertilization which play no part in polyspermy. In the latter case the factors which cause the entrance of the sperm are the ones involved.

In interpretating the data collected in Table VI. care must be exercised. In the first place it is not possible to control the concentration of the sperm so that relatively many more may not have been added to C3 eggs, for example, than to D3. It is also possible that one set may have been handled more than another, although precautions were taken to eliminate these sources of confusion. Previous investigators have found a great deal of variation in the responses of different lots of *Cumingia* sperm and eggs during development, and the factors governing the behavior of any given lot are rather elusive and uncertain. It has already been pointed out in this paper that these experiments should be carefully repeated before a final acceptance of the interpretations here given.

[1] Number of eggs counted was too small to permit an entirely trustworthy observation as to polyspermy.

The behavior of the extra sperm in the experiment, however, calls to mind the change in the activity of *Arbacia* fertilizin under the influence of X-radiation (Richards and Woodward, 1915). This secretion from *Arbacia* eggs possesses the power of activating sperm, and this property is affected by X-rays as follows: a slight radiation (2 min.) increases its activity, a long one (7 min.) lessens it, while an exposure of intermediate duration is practically without effect. Since a solution of *Arbacia* fertilizin in sea water (egg water) is definitely affected by irradiation, it is very probable that the substance which escapes from the eggs into the water would also be influenced by the irradiation before it passes out of the eggs.

The egg secretions of *Cumingia* and their properties have not been investigated, but Miss Cobb (see Woodward, '18, p. 464) "discovered that the eggs and egg water of *Cumingia* produce positive chemotactic response on the part of the sperm." Miss Sampson has found (unpublished) a secretion from *Cumingia* eggs which agglutinates its own sperm into very small groups of three of four sperm, and produces a very strong agglutination of *Arbacia* sperm.

In view of these facts and reasoning from the analogy, we are tempted to explain the facts shown in Table VI. by assuming that the fertilizin of *Cumingia* while yet in the egg was affected by the irradiation in such a manner as to bring about the results shown. The long radiations in each case where eggs were exposed, C15, and D15, show fewer extra sperm than the control which is to be expected if the long exposure decreases the activity of the substance as in the case of *Arbacia* fertilizin. Likewise in C3, a short exposure, more sperm entered into than the control eggs. This, too, is to be expected if the short irradiation activates the fertilizin. D3 represents the count of too few eggs to be dependable. In B3 and B15 where the sperm were radiated with the result that an increased number entered the egg after the short exposure and fewer than the control number are present, we may be dealing with a similar effect of the irradiation upon the sperm receptors (see Lillie, '19), although at the present time there is not even an analogy upon which to base such an assumption.

The irradiation of *Nereis* eggs (Packard) before fertilization frequently caused marked polyspermy, due to interference with the jelly formation about the egg. In *Nereis* the amount of jelly extruded from the egg at the time of fertilization is characteristically large; a similar explanation here is doubtfully possible in view of the smaller amount of jelly.

There is also other evidence that fertilization is affected by exposure to X-rays by means of a chemical change in the combining substance in the egg or the sperm. Richards ('15) and Packard ('16) have both shown the power of a short radiation in effecting a chemical change in proteids and some enzymes. Swartz ('08) found that exposure to radium is able to effect a chemical change in lethicin. Hertwig ('14) showed the direct effect of radiations of radium upon the chromatin content of the cell. These instances all serve to render plausible the explanation here suggested.

Summary.

1. A short radiation of the fertilized egg of *Cumingia* stimulates cell division for a time then retards it. A longer radiation produces less stimulation and a greater retardation.

2. A short or long sperm radiation before fertilization if not too intense affects the rate of cleavage in no way.

3. A short radiation of the unfertilized egg causes, when fertilized with normal sperm, a retardation in development, the production of abnormalities and complete inhibition of growth before the free-swimming larval stages are reached.

4. A short irradiation of both sperm and egg before fertilization results in a direct retarding of cleavage and in the production of abnormal embryos, most of them never reaching the free-swimming larval stage.

5. Eggs fertilized by sperm that have been subjected to a short exposure to X-rays result in a greater percentage of developing eggs than would normally occur. A long radiation produces an indifferent result.

6. An exposure to X-rays of the unfertilized egg results in a lesser percentage of developing eggs upon fertilization by normal sperm. No difference is evident due to time of exposure.

7. Eggs and sperm each exposed to the X-ray before mixing

results in a lesser percentage of developing eggs than when the eggs alone were irradiated before fertilization. No difference is evident due to time of exposure.

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